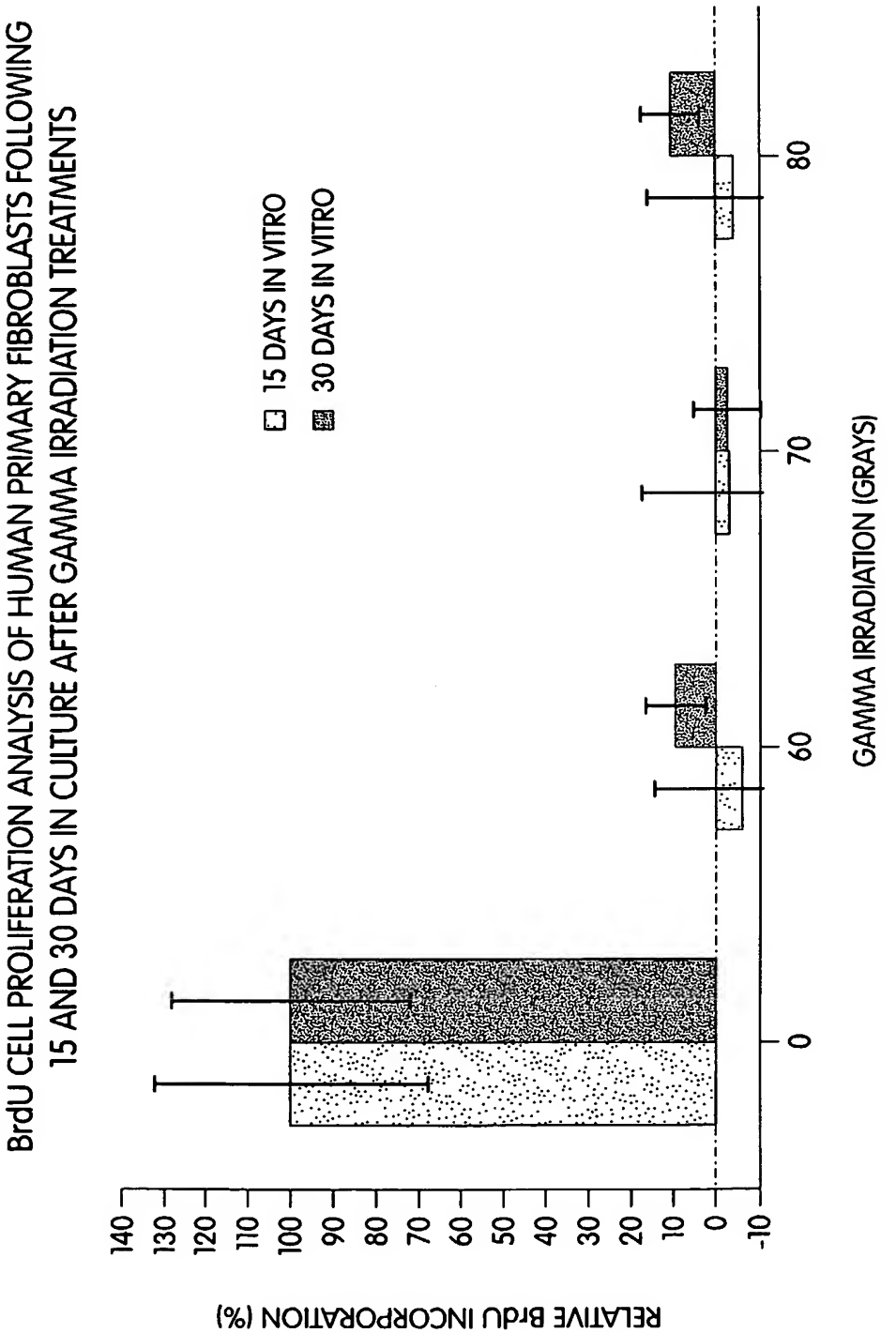
BrdU CELL PROLIFERATION ANALYSIS OF HUMAN PRIMARY FIBROBLASTS FOLLOWING 15 AND 30 DAYS IN CULTURE AFTER GAMMA IRRADIATION TREATMENTS
15 DAYS IN VITRO
30 DAYS IN VITRO
RELATIVE BrdU INCORPORATION (%)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
0
60
70
80
GAMMA IRRADIATION (GRAYS)

Fig. 1

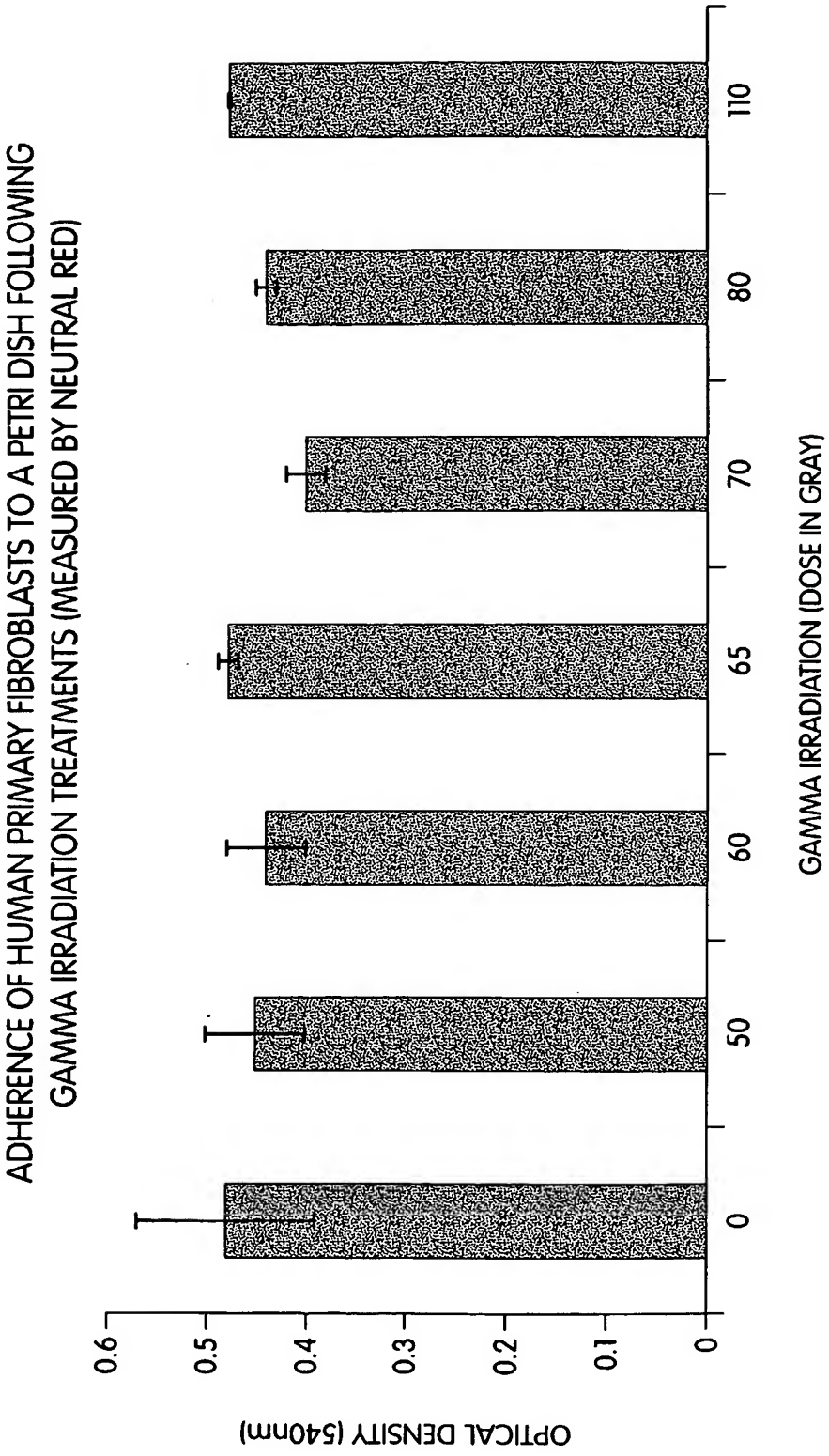
ADHERENCE OF HUMAN PRIMARY FIBROBLASTS TO A PETRI DISH FOLLOWING GAMMA IRRADIATION TREATMENTS (MEASURED BY NEUTRAL RED)
OPTICAL DENSITY (540nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
50
60
65
70
80
110
GAMMA IRRADIATION (DOSE IN GRAY)

Fig. 2

	THROMBIN 1/4		THROMBIN 1/8		THROMBIN 1/10		THROMBIN 1/20		THROMBIN 1/40		THROMBIN 1/80	
FIBRINOGEN 1/4	POLYMERIZATION TIME (SEC)	10	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	5	POLYMERIZATION TIME (SEC)	5	POLYMERIZATION TIME (SEC)	15	POLYMERIZATION TIME (SEC)	15
	CONSISTENCY	++	CONSISTENCY	++	CONSISTENCY	++	CONSISTENCY	++	CONSISTENCY	+	CONSISTENCY	++
	STRENGTH	+	STRENGTH	+	STRENGTH	+	STRENGTH	+	STRENGTH	+	STRENGTH	+
FIBRINOGEN 1/8	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20
	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	-	CONSISTENCY	++
	STRENGTH	-	STRENGTH	-	STRENGTH	+	STRENGTH	+	STRENGTH	-	STRENGTH	+
FIBRINOGEN 1/10	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	20
	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	-	CONSISTENCY	+
	STRENGTH	-	STRENGTH	-	STRENGTH	+	STRENGTH	+	STRENGTH	-	STRENGTH	+
FIBRINOGEN 1/20	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	15	POLYMERIZATION TIME (SEC)	15
	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	+
	STRENGTH	-	STRENGTH	-	STRENGTH	-	STRENGTH	+	STRENGTH	+	STRENGTH	+
FIBRINOGEN 1/40	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	20
	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	-	CONSISTENCY	+	CONSISTENCY	++
	STRENGTH	-	STRENGTH	-	STRENGTH	-	STRENGTH	-	STRENGTH	+	STRENGTH	+
FIBRINOGEN 1/80	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	30	POLYMERIZATION TIME (SEC)	20	POLYMERIZATION TIME (SEC)	15
	CONSISTENCY	-	CONSISTENCY	+	CONSISTENCY	++	CONSISTENCY	+	CONSISTENCY	+	CONSISTENCY	++
	STRENGTH	-	STRENGTH	+	STRENGTH	+	STRENGTH	+	STRENGTH	+	STRENGTH	+

Fig. 3

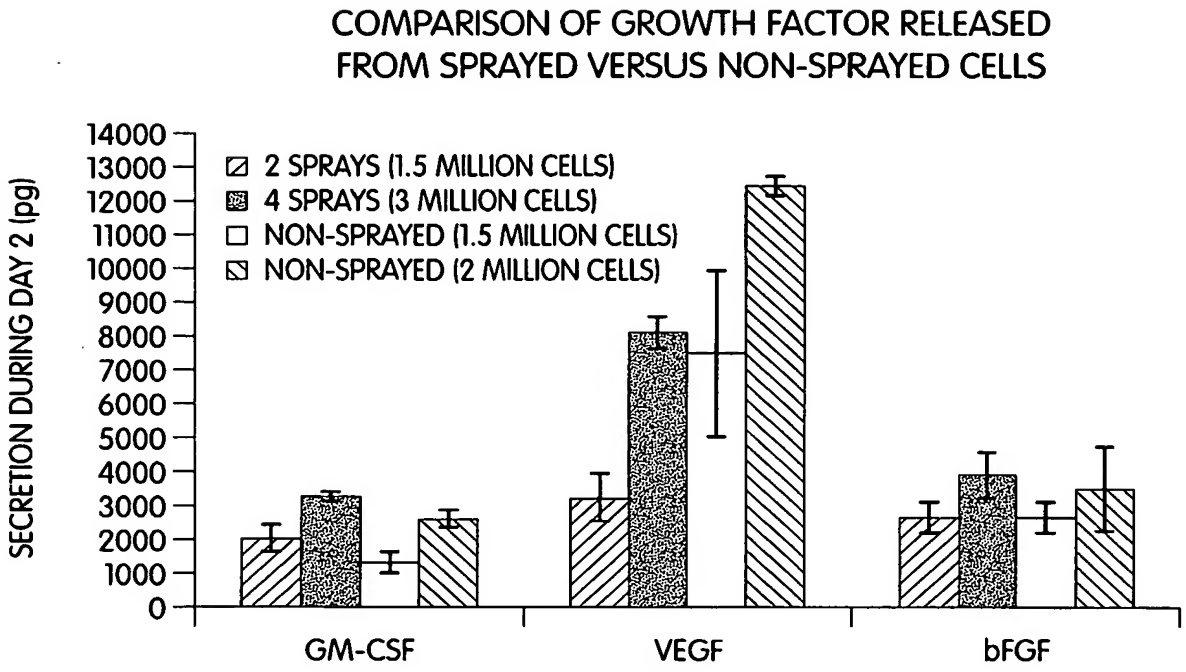

Fig. 4

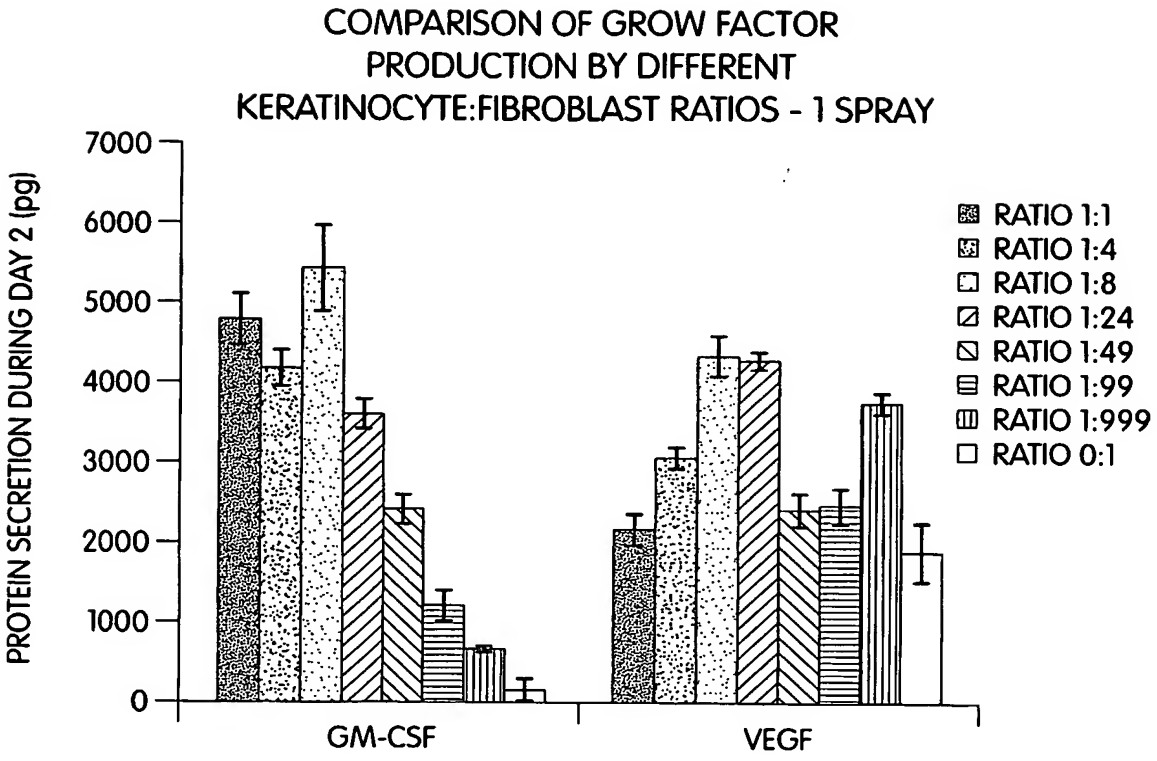
COMPARISON OF GROW FACTOR
PRODUCTION BY DIFFERENT
KERATINOCYTE:FIBROBLAST RATIOS - 1 SPRAY
PROTEIN SECRETION DURING DAY 2 (pg)
7000
6000
5000
4000
3000
2000
1000
0
GM-CSF
VEGF
RATIO 1:1
RATIO 1:4
RATIO 1:8
RATIO 1:24
RATIO 1:49
RATIO 1:99
RATIO 1:999
RATIO 0:1

Fig. 5A

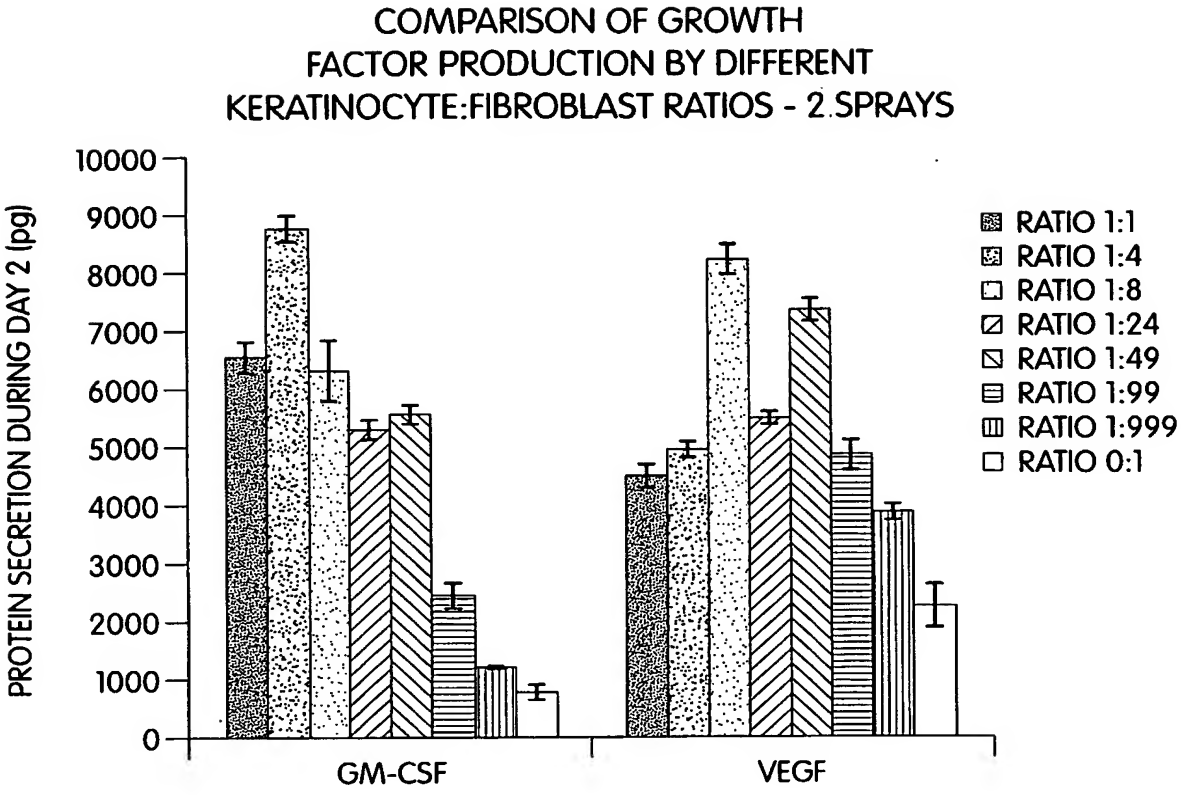

Fig. 5B

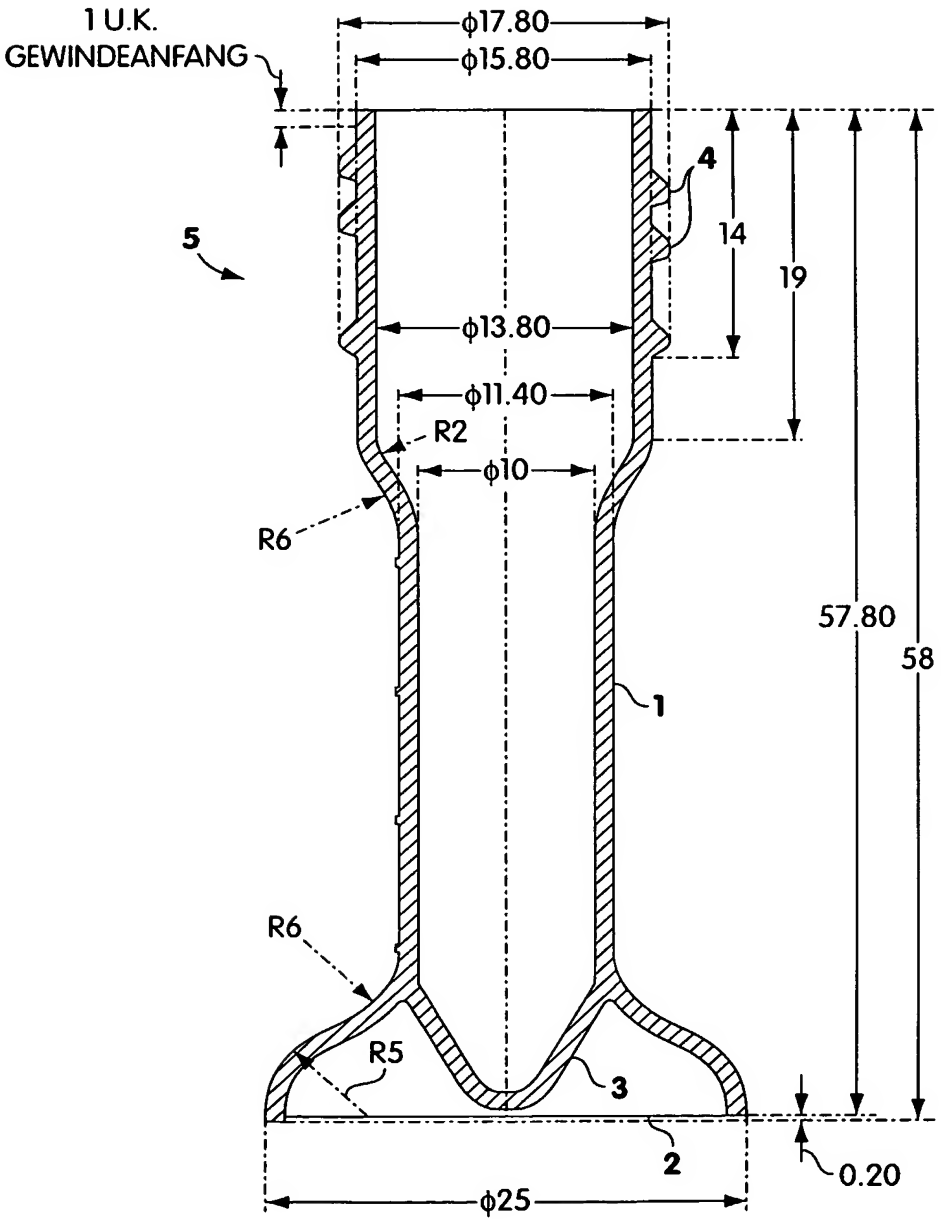
1 U.K.
GEWINDEANFANG
ϕ17.80
ϕ15.80
4
5
14
19
ϕ13.80
ϕ11.40
R2
ϕ10
R6
57.80
58
1
R6
R5
3
2
0.20
ϕ25

Fig. 6A

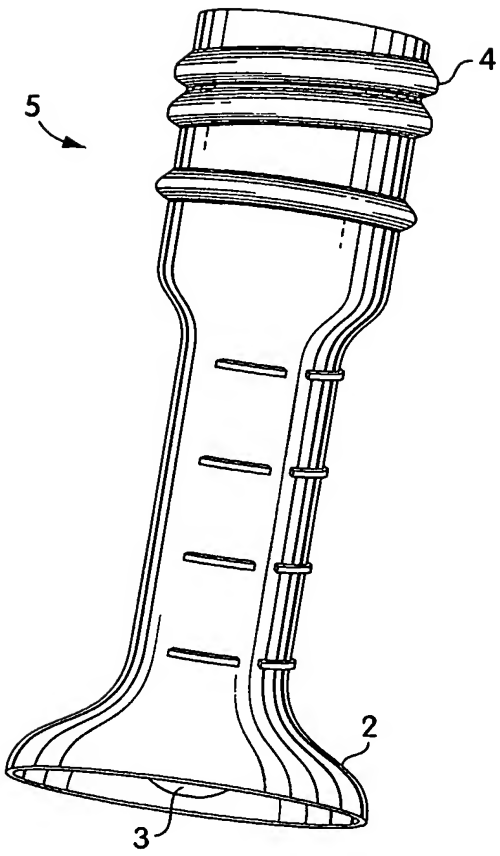

Fig. 6B

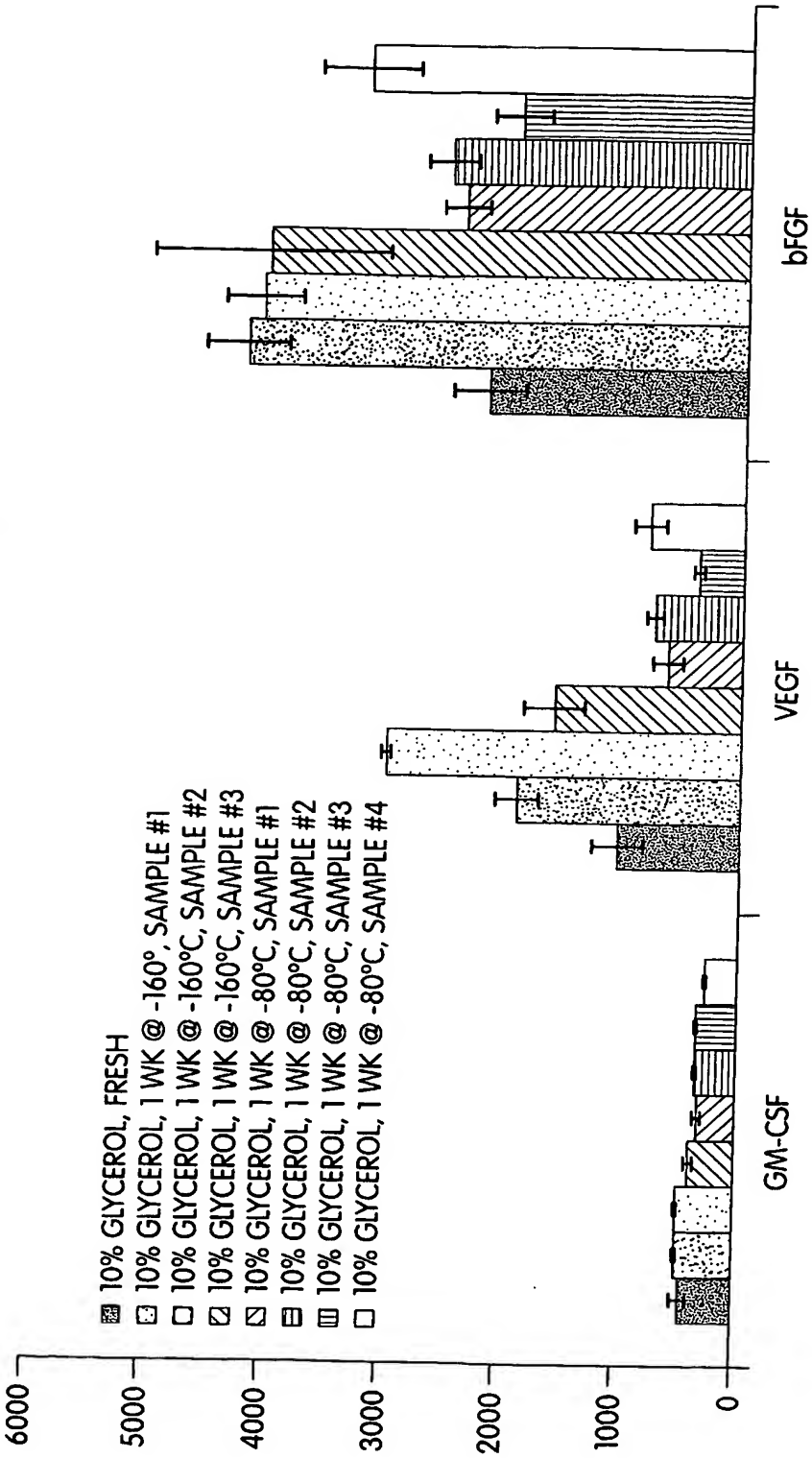
COMPARISON OF STORAGE AT -160°C VERSUS -80°C DURING 1 WEEK
10% GLYCEROL - KERATINOCYTE:FIBROBLAST RATIO (1:1), 10 MILLION CELLS/ml,
1 SPRAY (130 ul), 24 WELL
10% GLYCEROL, FRESH
10% GLYCEROL, 1 WK @ -160°, SAMPLE #1
10% GLYCEROL, 1 WK @ -160°C, SAMPLE #2
10% GLYCEROL, 1 WK @ -160°C, SAMPLE #3
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #1
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #2
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #3
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #4
SECRETION DURING DAY 2 (pg)
6000
5000
4000
3000
2000
1000
0
GM-CSF
VEGF
bFGF

Fig. 7

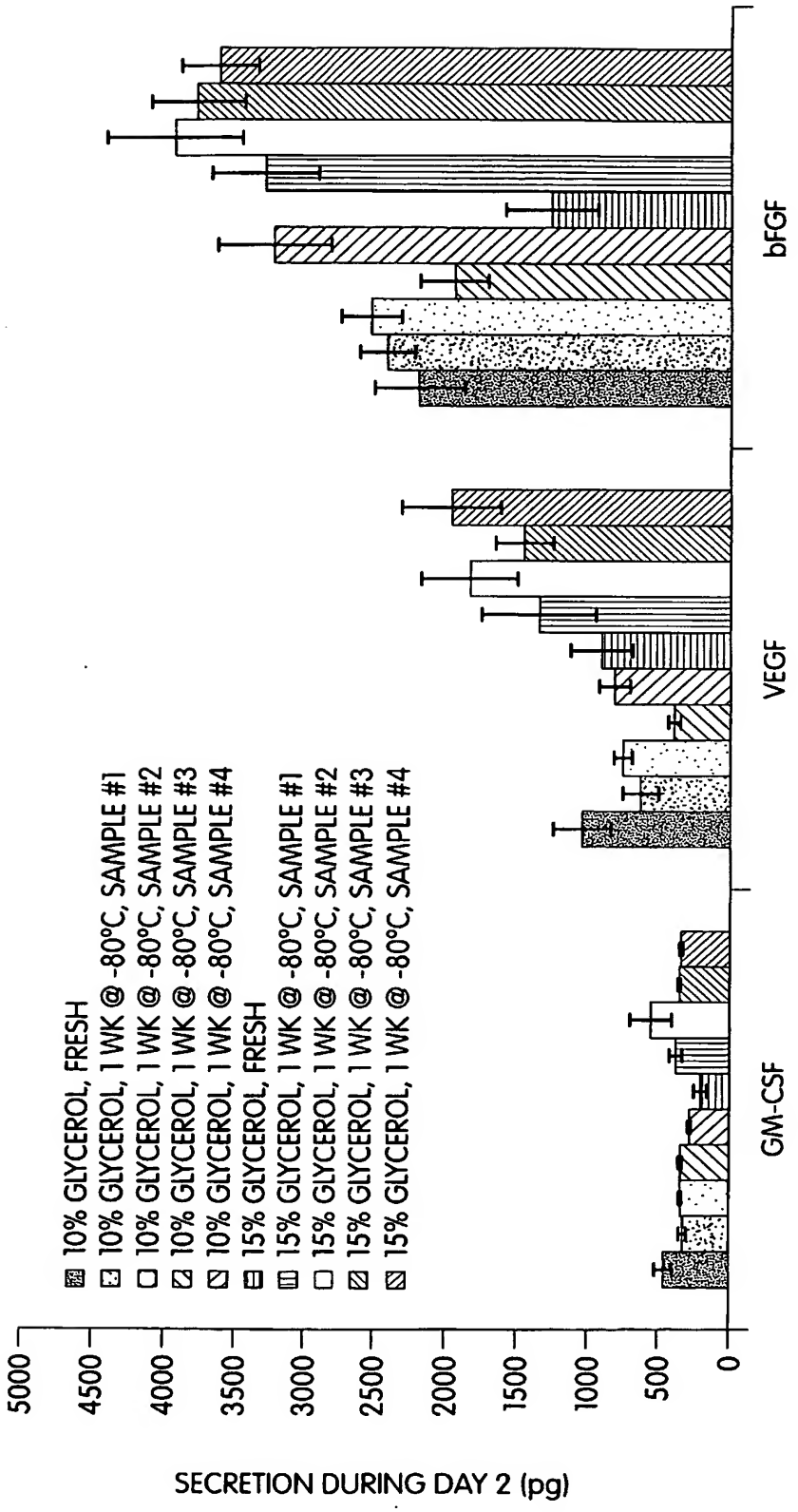
COMPARISON OF SECRETION AFTER 1 WEEK STORAGE AT -80°C
10% GLYCEROL VERSUS 15% GLYCEROL; 10 MILLION CELLS/ml
KERATINOCYTE:FIBROBLAST RATIO (1:1), 1 SPRAY (130 ul)
10% GLYCEROL, FRESH
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #1
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #2
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #3
10% GLYCEROL, 1 WK @ -80°C, SAMPLE #4
15% GLYCEROL, FRESH
15% GLYCEROL, 1 WK @ -80°C, SAMPLE #1
15% GLYCEROL, 1 WK @ -80°C, SAMPLE #2
15% GLYCEROL, 1 WK @ -80°C, SAMPLE #3
15% GLYCEROL, 1 WK @ -80°C, SAMPLE #4
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
GM-CSF
VEGF
bFGF
SECRETION DURING DAY 2 (pg)

Fig. 8

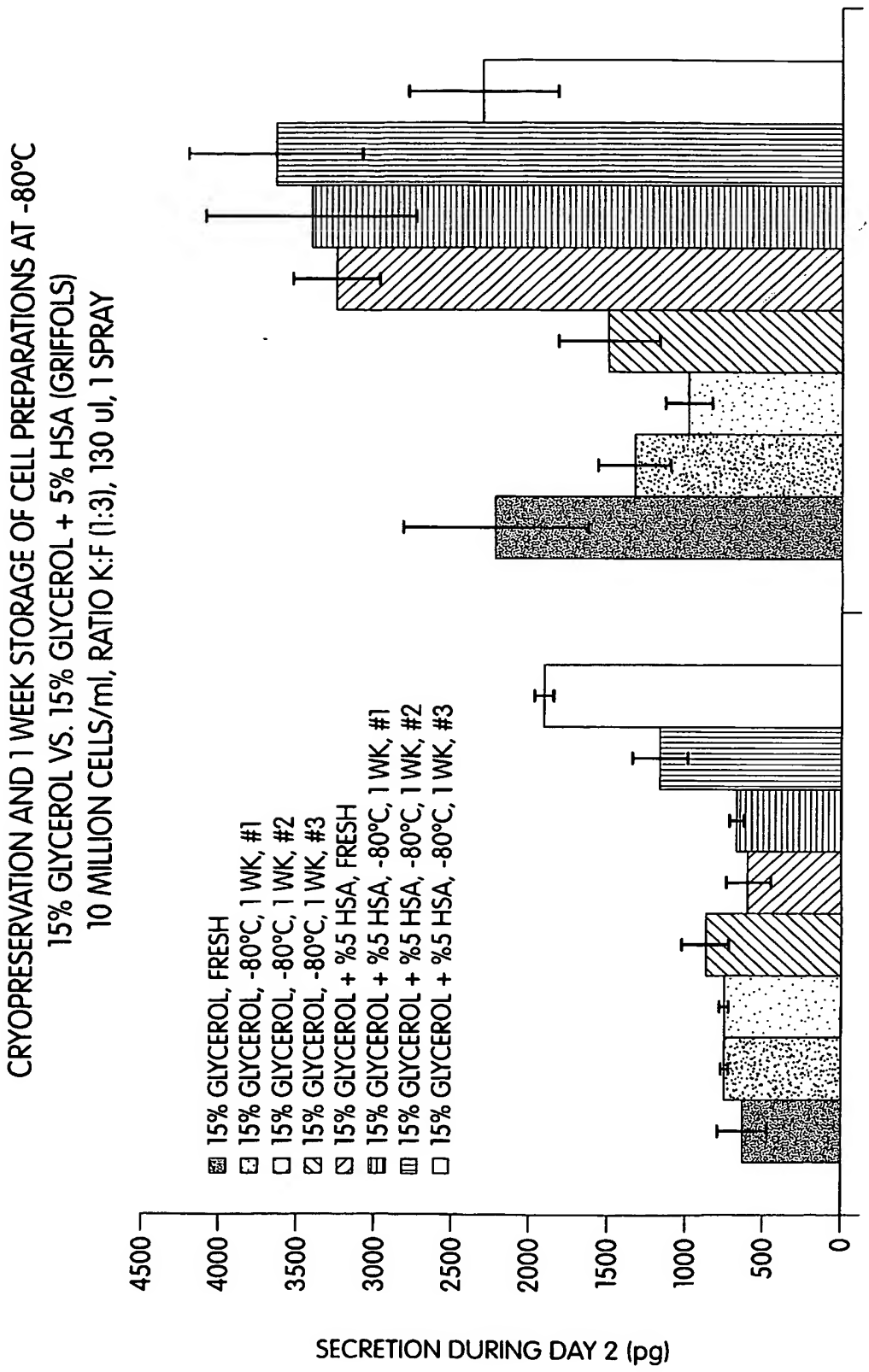

Fig. 9

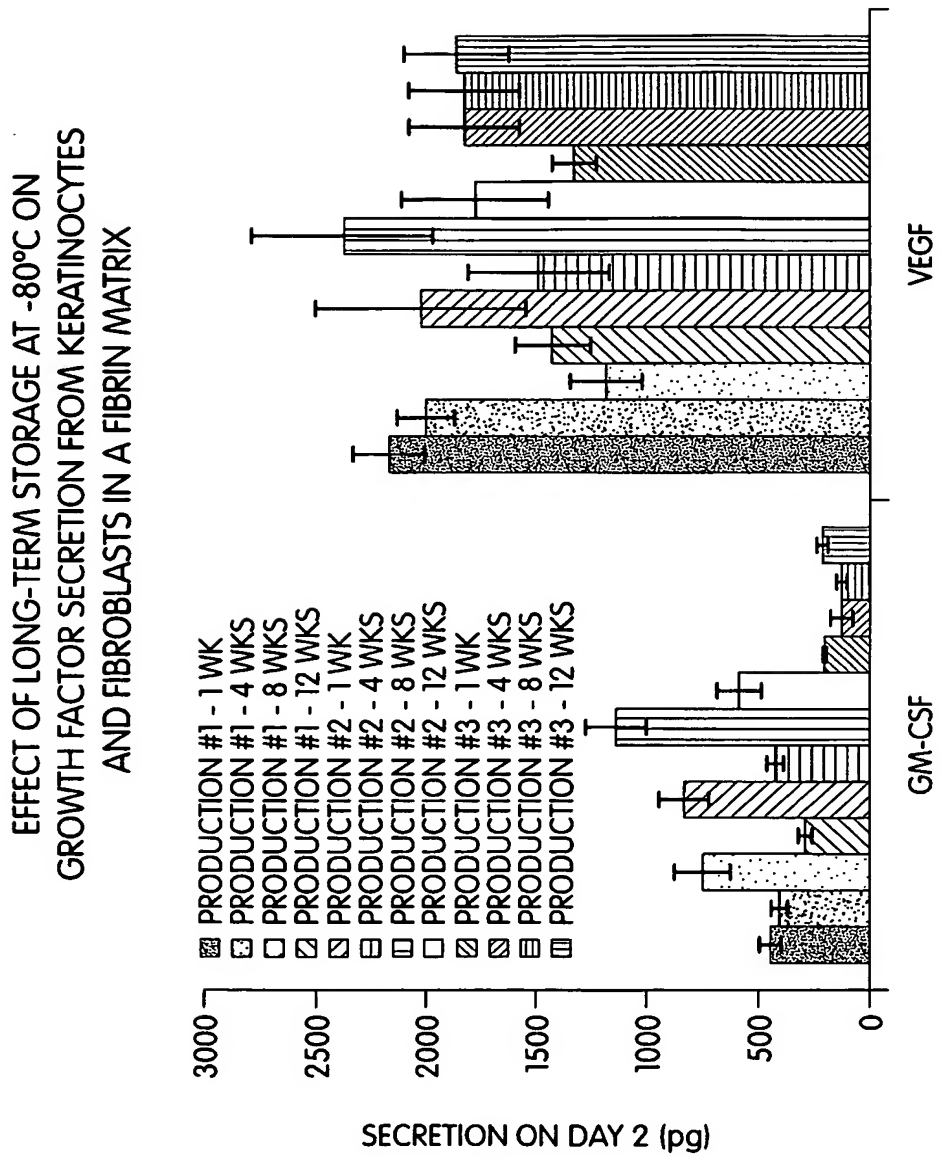
EFFECT OF LONG-TERM STORAGE AT -80°C ON
GROWTH FACTOR SECRETION FROM KERATINOCYTES
AND FIBROBLASTS IN A FIBRIN MATRIX
PRODUCTION #1 - 1 WK
PRODUCTION #1 - 4 WKS
PRODUCTION #1 - 8 WKS
PRODUCTION #1 - 12 WKS
PRODUCTION #2 - 1 WK
PRODUCTION #2 - 4 WKS
PRODUCTION #2 - 8 WKS
PRODUCTION #2 - 12 WKS
PRODUCTION #3 - 1 WK
PRODUCTION #3 - 4 WKS
PRODUCTION #3 - 8 WKS
PRODUCTION #3 - 12 WKS
3000
2500
2000
1500
1000
500
0
SECRETION ON DAY 2 (pg)
GM-CSF
VEGF

Fig. 10

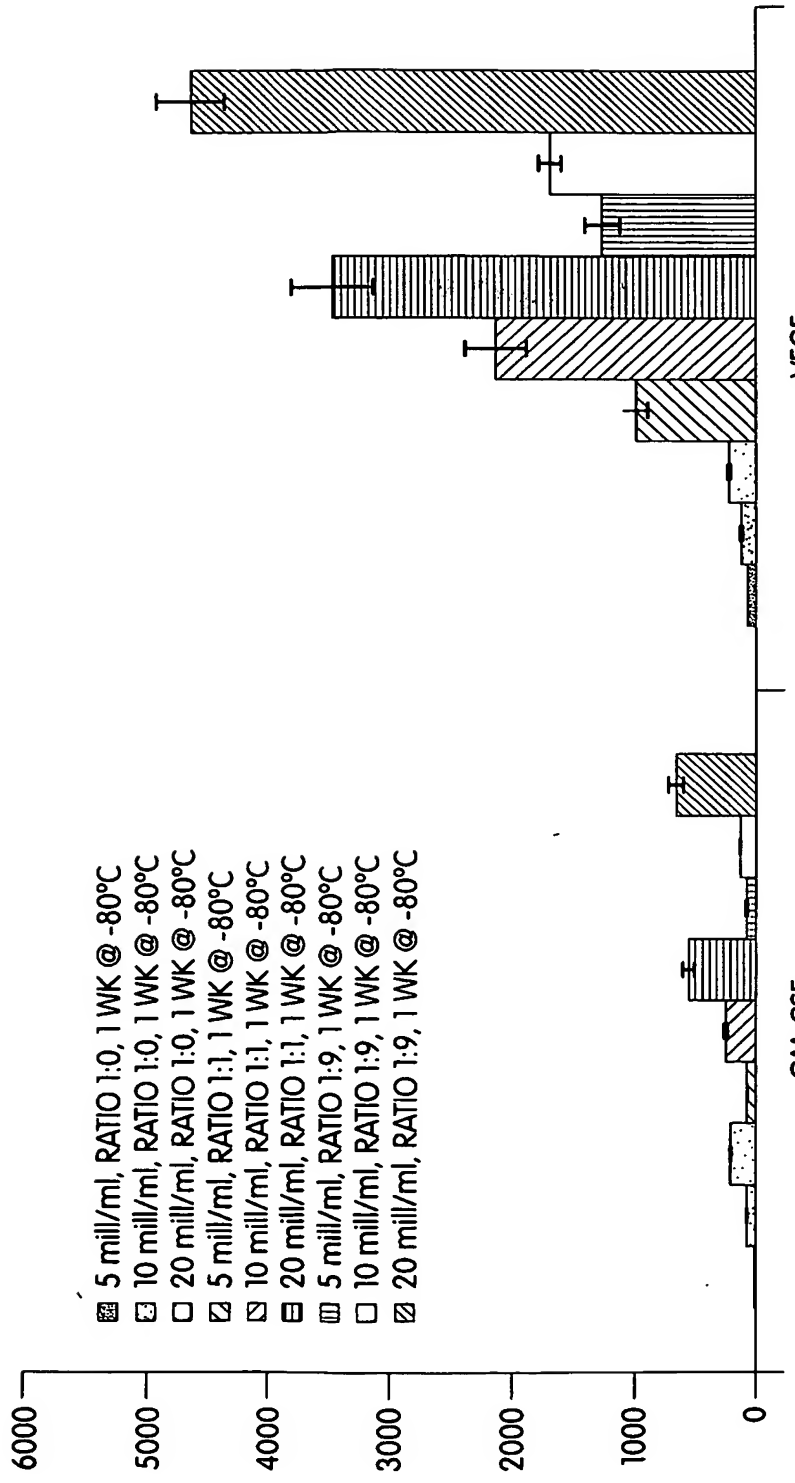
SECRETION BY THAWED CELL PREPARATIONS THAT WERE FROZEN AND STORED
FOR 1 WEEK AT -80°C; RATIOS OF KERATINOCYTES:FIBROBLASTS (1:0, 1:1, AND 1:9)
AT 5, 10 AND 20 MILLION CELLS/ml, 1 SPRAY (130 ul), 24 WELLS (n=15)
5 mill/ml, RATIO 1:0, 1 WK @ -80°C
10 mill/ml, RATIO 1:0, 1 WK @ -80°C
20 mill/ml, RATIO 1:0, 1 WK @ -80°C
5 mill/ml, RATIO 1:1, 1 WK @ -80°C
10 mill/ml, RATIO 1:1, 1 WK @ -80°C
20 mill/ml, RATIO 1:1, 1 WK @ -80°C
5 mill/ml, RATIO 1:9, 1 WK @ -80°C
10 mill/ml, RATIO 1:9, 1 WK @ -80°C
20 mill/ml, RATIO 1:9, 1 WK @ -80°C
SECRETION DURING DAY 2 (pg)
6000
5000
4000
3000
2000
1000
0
GM-CSF
VEGF

Fig. 11